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## Note

# The anti-inflammatory and analgesic activity of some benzimidazoles, and their ability to protect erythrocytes from hemolysis by silica powder

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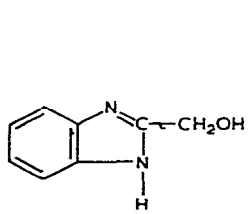
We have shown[1] that methanesulfonylation of 2-benzimidazolemethanol (**1**) in chloroform containing a limited proportion of pyridine affords 2-(chloromethyl)-1-(methylsulfonyl)benzimidazole (**2**). However, when compound **1** was treated with methanesulfonyl chloride in pyridine, only, at room temperature, only a small proportion of amorphous material was obtained on decomposing the reaction mixture with water. It was suggested[1] that a quarternary compound might have been produced, and that this was soluble in water. Shaw and co-workers[2] reported that a quarternary compound was produced when a plant-cell promoting-factor, zeatin (**3**), was treated with methanesulfonyl chloride in pyridine. Grimaldi and Day[3] prepared quarternary compounds by treating 2-(chloromethyl)-1-(ethoxycarbonyl)benzimidazole with 5-(2-hydroxyethyl)-4-methylthiazole in absolute ethanol.



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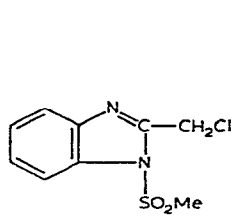

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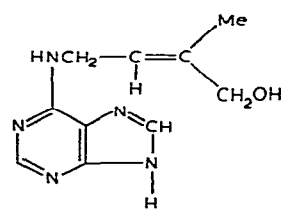

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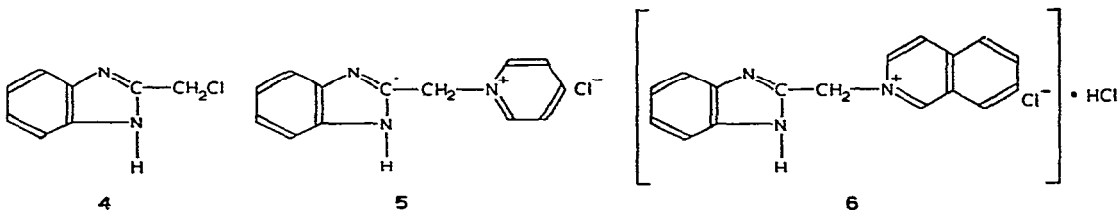
We now report that 2-(chloromethyl)benzimidazole (**4**) readily forms the quarternary compound **5** on treatment with pyridine in boiling 1,4-dioxane. Isoquinoline also reacts readily with **4** in boiling 1,4-dioxane, to yield a quarternary compound which was converted into the hydrochloride (**6**); this was easily purified by recrystallization from methanol.

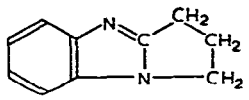


4 5 6

Further studies were made with a hemolysis test-system designed originally to investigate the *in vitro* action of silica powder and various forms of asbestos fibers on erythrocytes[4–6]. As silica has negative surface-charges, it seemed feasible that quarternary compounds might be effective in blocking the hemolytic action of silica. Accordingly, compounds **5** and **6** were tested for their ability to prevent, or retard, the marked hemolytic action of finely divided silica powder. (The results of the hemolysis tests had shown that silica powder by itself is 92% hemolytic.) Compound **5** was completely inactive in preventing this hemolysis, the degree of hemolysis being 96% at the end of the experiment. By contrast, the hemolytic activity of silica decreased to 31% after it had been pretreated with **6**, which thus protected to the extent of 61%. In tests with both **5** and **6**, the final pH of the supernatant solution (before spectrophotometric reading) was 7.2. Interestingly, the 61% protection afforded by **6** is similar to that (59%) given by trisodium (ethylenedinitrilo)tetraacetate against hemolysis by chrysotile asbestos[4,6], but less effective than poly(2-vinylpyridine 1-oxide) against silica powder[7] (89–92%).

Seeman and Weinstein[8] found that low concentrations of several tranquilizers and antihistamines (phenothiazines, reserpine, and haloperidol) protect or stabilize erythrocytes against hypotonic and mechanical hemolysis. Local anesthetics[9] (dibucaine hydrochloride, tetracaine hydrochloride, and others) and a wide variety of surface-active and lipid-soluble substances[10] are also active in this regard. In addition, all of the clinically active, nonsteroidal, anti-inflammatory drugs tested by Inglot and Wolna[11] were active at low concentrations in protecting erythrocytes against hypotonic hemolysis. Because of a possible connection between compounds that have anti-inflammatory activity and those that can protect membranes from lysis, Lederle Laboratories tested compounds **5** and **6** in their phenyl-*p*-quinone, anti-writhing screen (PQR), a test system used to detect potential anti-inflammatory, or potential analgesic, agents. Dr. J. Denton[12] of Lederle informed us that **5** is inactive in the PQR test. However, compound **6** showed activity in this test system. Subsequent testing showed that **6** is also active as an analgesic agent in the rat-paw pain test, although the activity is low. Compound **6** also shows some anti-inflammatory activity. These results parallel those we have obtained with the silica hemolysis test-system.

It is well known that benzimidazole derivatives may be effective analgesics and muscle relaxants[13]. During the course of this investigation, we became interested in 2,3-dihydro-1-*H*-pyrrolo[1,2-*a*]benzimidazole (**7**), a compound readily obtained by



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treating 2-benzimidazolepropanol with thionyl chloride in *N,N*-dimethylformamide[14]. Dr. E. May of the National Institute for Arthritis, Metabolism, and Digestive Diseases, Bethesda, Md. 20014, U. S. A., examined compound **7** in the mouse hot-plate test-system, and found[15] a low order of analgesic activity. The $ED_{50}$ was 66 mg/kg; in the same test-system, codeine had an $ED_{50}$ of 7.5 mg/kg, and morphine, of 1.2 mg/kg. Lederle Laboratories found that compound **7** had activity in the PQR test-system. Further investigation showed that this compound had motor-depressant activity as a central nervous system agent, with the motor-depressant dose being ~10 mg/kg. However, it did not show significant activity in the rod-walking and in the strychnine-shock tests[12].

## EXPERIMENTAL

*General.* — Unless otherwise stated, melting points are uncorrected. Microanalyses were performed by Dr. W. Alford and his associates at the National Institute for Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20014, U. S. A.

*Reaction of 2-(chloromethyl)benzimidazole* (**4**) *with pyridine.* — Pyridine (10 ml) was added to a hot solution of **4** (3.7 g) in dry 1,4-dioxane (40 ml), and the solution was boiled under reflux; after 15 min, a dark oil separated from solution, and this oil solidified on shaking the mixture. Boiling under reflux was then continued for 1.5 h, the suspension was cooled, and the yellow solid (5.2 g) was filtered off and dried *in vacuo*. On recrystallization from methanol (decolorizing carbon), a faintly colored, quaternary salt (3.5 g), m.p. 230°, was obtained. A second recrystallization from methanol afforded white needles of **5**, m.p. 230°.

*Anal.* Calc. for $C_{13}H_{12}ClN_3$: C, 63.55; H, 4.92; Cl, 14.43; N, 17.10. Found: C, 63.57; H, 5.18; Cl, 14.32; N, 16.76.

*Reaction of 2-(chloromethyl)benzimidazole* (**4**) *with isoquinoline.* — Isoquinoline (12 ml) was added to a hot solution of **4** (4.8 g) in 1,4-dioxane (50 ml). After the mixture had been boiled under reflux for 15 min, an oil separated out. Boiling was continued for 1.5 h, and the mixture was kept overnight at room temperature. The suspension was reheated to boiling, and the dark solid was filtered off from the hot mixture, washed with a small volume of 1,4-dioxane, and dried *in vacuo*. It was then crushed, suspended in 1,4-dioxane (40 ml), and the suspension boiled under reflux for a few minutes, cooled, and the solid filtered off, and dried (yield, 7.6 g). A portion (7.0 g) of this product was dissolved in warm, concentrated hydrochloric acid (30 ml), and the solution was evaporated to dryness under diminished pressure. The residue was triturated with acetone, and the crystals of the hydrochloride of the quaternary

compound were filtered off, washed with acetone, and dried. Recrystallization from methanol (decolorizing carbon) afforded faintly colored crystals (4.8 g) of **6**, m.p. 242° (dec.).

*Anal.* Calc. for $C_{17}H_{15}Cl_2N_3$: C, 61.46; H, 4.55; Cl, 21.34; N, 12.65. Found: C, 61.45; H, 4.49; Cl, 21.10; N, 12.46.

*Investigation of compounds* **5** *and* **6** *in a hemolysis system using silica powder.* — The activity of the silica ["Fransil" colloidal silica (X5663), uncompressed, particle diameter up to 0.3 $\mu$m] was determined by adding, in duplicate, samples (50 mg) of veronal-buffered saline (3 ml) and a 2%, standardized suspension of washed, sheep erythrocytes, also in veronal-buffered saline (1 ml). For studies of any preventive action of compounds **5** and **6**, 0.05% solutions (2 ml) of each compound in veronal buffer were added to silica powder (50 mg) in buffer (2 ml); that is, 1 mg of test compound per 50 mg of silica. The mixtures were well agitated (by mechanical shaking on a "Vortex" rotary mixer) before being incubated for 1 h in a water bath at 37°, with gentle shaking every 15 min. After incubation, the suspensions were centrifuged, and the supernatant liquor was discarded. The treated silica was washed 3 times with veronal-buffered saline (to remove any unadsorbed compound). Buffer (2 ml) and erythrocyte suspension (2 ml) were added, and then the standard procedures for testing of compounds against hemolysis by silica were followed[4]. At the end of 1 h of incubation, followed by centrifugation, the degree of hemolysis was determined by measuring, at 541 nm, the amount of hemoglobin in the supernatant liquor[4]. As controls, erythrocyte suspension (2 ml) and distilled water (2 ml) were used in duplicate, and, as blank samples, erythrocyte suspension (2 ml) and buffer (2 ml); these served to check for possible mechanical disruption of erythrocytes during shaking. Both the control and the blank samples were treated in exactly the same way as the test samples.

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